Immunology, pathophysiology, and treatment of malaria

Walter R. Weiss, MD, MPH, and Stephen L. Hoffman, MD, DTMH

Naval Medical Research Institute, Bethesda, Maryland, USA

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Malaria is a major world health problem with hundreds of millions of cases and millions of deaths per year. Research on malaria concentrates on *Plasmodium falciparum*: this parasite is the major cause of the fatal disease. This review highlights research in the areas of vaccine development, pathophysiology of disease, drug resistance, and drug therapy.

Vaccine development

Vaccine research in malaria targets three stages of the parasite life cycle: 1) the preerythrocytic stages, encompassing sporozoite and liver stage parasites; 2) the blood-stage cycle of clinical infection; and 3) the sexual stages of the parasite in the mosquito. The goal of the preerythrocytic vaccines is sterile immunity against the early stages of infection to prevent the symptomatic blood stage disease. Vaccines aimed at the malaria blood stages could seek sterile immunity as well, but might also be effective if they contained the infection and prevented the most severe sequelae. Vaccines directed at the sexual stages prevent development in the mosquito. They do this by inducing human antibodies against parasite antigens expressed in the insect vector. These antibodies are ingested by the mosquito with the infected blood meal and interfere with parasite development.

Preerythrocytic vaccines

The most studied antigen from the preerythrocytic phases of the life cycle is the circumsporozoite protein, which covers the surface of the sporozoite. The circumsporozoite antigen repeat motif is the predominant epitope recognized by antibodies in the serum of sporozoite immunized animals. Transfer of monoclonal antibodies directed at this repeat epitope can protect mice against infection by sporozoites. However, polyclonal serum antibodies are much less protective than the monoclonal antibodies. Do Rosario *et al.* [1] has now shown that immune serum may actually aid the parasite during its development in the mosquito. They infected mosquitoes with *P. falciparum* and let them feed on blood containing antisporozoite antibodies. The number of sporozoites per mosquito more than doubled. Furthermore, *in vitro* liver cell invasion by these sporozoites was now unaffected by immune serum that otherwise neutralized sporozoites. The authors concluded that the effects on transmission of malaria should be considered when assessing any candidate vaccine.

Recent work has focused on the responses of T cells to the circumsporozoite protein because it now appears that immune T cells can kill pre-erythrocytic forms of malaria. Hoffman *et al.* (*Science* 1987, 237:639) had previously reported results from a prospective study of adults in Kenya that showed no correlation of anti-circumsporozoite serum antibody with protection from malaria. Now Hoffman *et al.* [2] report that in this group, there was a correlation of protection with T-cell proliferative responses to a short segment of the *P. falciparum* circumsporozoite protein. Although this study was small, it is perhaps significant that this same segment contains an epitope recognized by cytotoxic T cells, which are important immune effector cells in the animal models of sporozoite immunity.

Romero *et al.* [3] showed that T cells reactive with the circumsporozoite molecule can protect mice against sporozoite infection. They first identified a cytotoxic T-cell epitope on the *Plasmodium berghei* circumsporozoite protein and then raised $CD8^+$ T-cell clones against this short peptide. Transfer of several of these clones protected mice against sporozoite challenge. Very large numbers of cells were needed to protect these animals, but it is encouraging that the circumsporozoite protein contains potentially protective epitopes for T-cell immunity.

Hoffman *et al.* [4], in another paper, explored the role of $CD8^+$ T cells in the immune response to liver stages of malaria. They reported that immune mice develop cellular infiltrates in their livers after sporozoite chal-

Abbreviation

TNF—tumor necrosis factor.

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lenge. They transferred immune spleen cells, and were able to find similar infiltrates in recipient animals after challenge. Depleting immune spleens of $CD8^+$ cells before transfer abolished the infiltrates. Immune spleen cells could also inhibit parasite growth in cultured liver cells. These papers by Hoffman *et al.* [3] and Romero *et al.* [4] emphasized the importance of T-cell responses in protective immunity to the early stages of malaria infection. However, much remains to be learned about antigens and immune mechanisms before a vaccine based on T-cell immunity can be a reality in humans.

Blood-stage vaccine antigens

A large research effort aims at vaccine against the blood stages of malaria. The symptoms and lethal complications of malaria begin when the parasite ruptures from the liver and starts its cycles of erythrocyte infection. A vaccine that would mute or completely prevent this stage of the infection is thus very attractive. Furthermore, there is evidence from epidemiologic and clinical studies that some natural immunity to blood stage infections is acquired by persons living in malaria endemic areas. Several papers published this year dissected the immune responses to candidate malaria blood stage antigens.

Troy-Blomberg *et al.* [5] studied T- and B-cell responses to Pf155/RESA. This antigen is deposited on the erythrocyte surface by invading *P. falciparum* merozoites, and antibodies to it will block invasion *in vitro*. Fourteen synthetic peptides, corresponding to predicted T-cell epitopes, were tested for reactivity with cells and serum from Gambians living in a malarious area. Two peptides stimulated T-cell responses from 61% and 66% of the 46 donors, while ~2% responded to crude whole Pf155 antigen. Serum antibodies likewise reacted with many peptides, and for individual donors, there appeared to be an inverse correlation between B- and T-cell responses to the Pf155/RESA repeat regions. T-cell reactivity was maintained even after the season of malaria transmission, while antibody titers waned. This work provides the immunologic groundwork for designing a synthetic vaccine based on the Pf155/RESA sequences.

Burns *et al.* [6] studied another merozoite antigen in the *Plasmodium yoelii* rodent model. The major merozoite surface antigen coats the merozoite and passive transfer of monoclonal antibodies to this antigen protect mice against *P. yoelii* blood infection. Using an *Escherichia coli* expression system, the epitope of this protective monoclonal was mapped to a cysteine-rich fragment of the precursor major merozoite surface antigen. The epitope was destroyed by reduction and presumably is conformationally dependent on cross-linking of the cysteines. This region is highly conserved between malaria species, including *P. falciparum*, and is another promising candidate antigen for a blood stage malaria vaccine.

Marsh *et al.* [7] studied antibodies to blood stage antigens of *P. falciparum* in relation to parasitemia and disease. They followed 134 Gambian children and found that only the response to parasite-dependent red blood cell neoantigens, as measured by erythrocyte agglutination, was associated with lower incidence of clinical malaria. Interestingly, children reacting to these antigens had less illness but the same parasite levels as their nonreactive peers. This finding underlines the dichotomy between infection and clinical disease in malaria and hints that a vaccine to prevent illness may not have to produce sterile immunity.

Transmission-blocking vaccine antigens

Transmission-blocking vaccines are designed to kill malaria in the mosquito. Human antibodies directed against the sexual stages of the parasite are ingested by the mosquito and react with the developing stages in the mosquito gut. Kaslow *et al.* [8] studied one such target antigen, Pfs25. He sequenced the *Pfs25* gene from eight geographically distinct isolates of *P. falciparum* and found that they were identical except for one example of a single conservative amino acid change. This is encouraging as variation of malaria antigens is notorious. Protective transmission-blocking antibodies against this antigen should be effective against all strains.

Pathophysiology of malaria

Adhesion molecules

In *P. falciparum*, the mature stages of infected erythrocytes do not circulate freely but bind to capillary and venular epithelium. This prevents the the parasitized cells from being filtered by the spleen, and also may contribute to the vascular plugging that is the hallmark of cerebral malaria. Thus, the molecular basis of the adhesion of parasitized cells is of great interest.

Erythrocytes infected by *P. falciparum* have structures called "knobs" at their surface, and it was thought that these knobs were the site of binding to endothelium. Udomsangpetch *et al.* [9] discovered a knobless *P. falciparum* that retains its adherent properties in *in vitro* assays. Thus, the knob is merely a correlate of the true adherence molecules on the surface of the parasitized erythrocyte. The nature of the true adhesion molecule remains unknown.

Two naturally occurring molecules on endothelium, thrombospondin and CD36, have been proposed as host ligands for the parasite adhesion molecules. Sherwood *et al.* [10] found that purified thrombospondin could bind parasitized erythrocytes, but that binding to melanoma cells *in vitro* did not correlate with thrombospondin on their surface. Oquendo *et al.* [11] cloned the human CD36 gene and showed that transfected cells were able to bind parasitized erythrocytes. Ochenhouse *et al.* [12] purified CD36 from platelets and found that plastic coated with this product bound parasitized erythrocytes. Most significantly, addition of sol-

uble CD36 was then able to reverse the binding. Treatment for cerebral malaria using analogues of CD36 may thus be a possibility in the future.

Cytokines in severe malaria

Encephalopathy due to *P. falciparum* is a major cause of mortality and is thought to be due to vascular damage in the brain. As mentioned earlier, mature parasitized erythrocytes adhere to and clog small vessels in the brain, and may be the cause of the vascular lesion. An intriguing alternative hypothesis is that the vascular lesions are not due solely to erythrocyte plugging, but involve the toxic effects of a circulating cytokine, tumor necrosis factor (TNF). Some strains of mice infected with rodent malariae develop cerebral malaria and vascular lesions without plugging by infected erythrocytes. Instead leukocytes pack the damaged vessels. This lesion in mice can be prevented by *in vivo* treatment with antibody to the lymphocyte CD4 antigen or by antibody to TNF.

Grau *et al.* has published two new papers on TNF in malaria. In the first [13], cerebral malaria in the mouse was prevented by treatment of animals with antibodies to interferon-γ, which inhibited TNF production by macrophages. His model of immunopathology in mice is now that $CD4^+$ lymphocytes release lymphokines, including interferon-γ. These lymphokines induce TNF production from macrophages, and TNF is responsible for the lesions in the cerebral vessels.

In the second paper Grau *et al.* [14] related TNF levels to cerebral malaria in humans. They reported on a study of 65 severely ill Malawian children, and showed that serum TNF levels on hospital admission were high and correlated with young age, high parasite counts, hypoglycemia, and death. Thus, TNF may be involved in human as well as rodent cerebral malaria. This opens the exciting prospect of anti-lymphokine therapy for cerebral malaria, an otherwise poorly treatable condition.

Mechanisms of drug resistance

Resistance of *P falciparum* to chloroquine has become a major therapeutic problem. It appears that the parasite has evolved a mechanism to excrete the drug, which renders it insensitive to normal therapeutic drug levels. Calcium channel blocking drugs inhibit this excretion, and the parasite once again becomes sensitive to chloroquine. This reversal by calcium channel blockers is similar to multidrug resistance in mammalian tumors, in which P glycoprotein pumps chemotherapeutic agents out of cells making the tumor drug resistant. Calcium channel blockers inhibit the pump and restore drug sensitivity.

Two groups reported finding a gene in *P. falciparum* that is an analogue of the mammalian multidrug resistance gene [15,16]. This gene, *Pfmdr*, exists in all parasite strains but has a larger number of copies in some chloroquine-resistant parasites. Some resistant strains also have an increased amount of translated messenger RNA from the Pfmdr gene. This is all consistent with Pfmdr being the mechanism of chloroquine resistance in at least some instances.

Progress has also been made in understanding the mechanism of drug resistance to pyrimethamine [17]. This drug is an inhibitor of dihydrofolate reductase, an important enzyme in nucleic acid synthesis. Using genetic crosses between resistant and susceptible strains of *P. falciparum*, resistance to pyrimethamine was localized to chromosome 4 where the dihydrofolate reductase gene is situated. Resistance correlated with a single point mutation producing an asparagine at position 108, which the authors believe inhibits the binding of pyrimethamine. This knowledge of the mechanisms of drug resistance may lead to modifications that will increase the effectiveness of these antimalarials.

Treatment of severe malaria

Although mild cases of malaria respond to oral therapy, treatment of severe malaria can be complex because the side effects of therapies may exacerbate the hypoglycemia and circulatory collapse that is caused by malaria itself. Three papers addressed the pharmacology of quinine, chloroquine, and quinidine with an eye to minimizing their side-effects.

Molyneux *et al.* [18] studied dose rates of intravenous quinine in children with cerebral malaria. They found that quinine-induced insulin release and hypoglycemia could be avoided if the drug was infused over 3 hours instead of 1.

White *et al.* [19] reported on various routes and dosages of chloroquine in children with severe malaria. He studied intravenous, subcutaneous, intramuscular, and nasogastric methods of drug delivery, and found adequate serum drug levels achieved by all routes. Hypotension occurred most commonly in the higher dose parenteral regimens, although resolution of parasitemia and fever was similar in all groups. The authors recommended that when possible, chloroquine should be given by intravenous infusion but may also be given safely by multiple frequent injections.

Miller *et al.* [20] reviewed the US experience treating patients with severe malaria, using intravenous quinidine and exchange transfusion. Continuous intravenous quinidine was effective in decreasing parasite levels, and cardiac abnormalities, tinnitus, and vomiting were associated with elevated serum drug levels in a few cases. Exchange transfusion of up to 10 units of blood dropped parasite counts quickly. They recommend a protocol using both modalities where adequate hospital resources are available.

Annotated references and recommended reading

- • Of interest
- •• Of outstanding interest

1. • Do Rosario VE, Appiah A, Vaughan JA, Hollingdale MR: ***Plasmodium falciparum*: administration of anti-sporozoite antibodies during sporogony results in production of sporozoites which are not neutralized by human anti-sporozoite protein vaccine serum.** *Trans R Soc Trop Med Hyg* 1989, 83:305–307.

Feeding previously infected mosquitos on blood containing anti-sporozoite antibodies increased the number of sporozoites produced. In addition, these sporozoites could not be neutralized by human serum with anticircumsporozoite antibody. The effect of anti-sporozoite vaccines on transmission of malaria must be considered before any vaccine is widely used.

2. • Hoffman SL, Oster CN, Mason C, Beier JC, Sherwood JA, Ballou WR, Mugambi M, Chulay JD: **Human lymphocyte proliferation response to a sporozoite T-cell epitope correlates with resistance to falciparum malaria.** *Immunol* 1989, 142:1299–1303.

Resistance to reinfection with *P. falciparum* correlated with T-cell proliferative responses to a peptide fragment from the circumsporozoite protein. This indicates that T-cell immunity in humans is important in fighting off the preerythrocytic stages of malaria.

3. •• Romero P, Maryanski JL, Corradin G, Nussenzweig RS, Nussenzweig V, Zavala F: **Cloned cytotoxic T cells recognize an epitope in the circumsporozoite protein and protect against malaria.** *Nature* 1989, 341:323–326.

Cytotoxic T-cell clones were raised against a peptide epitope from the *P. berghei* circumsporozoite protein. Some of these clones were transferred to mice and protected them from sporozoite challenge. The circumsporozoite protein thus contains a protective T-cell epitope.

4. •• Hoffman SL, Isenbarger D, Long GW, Sedegah M, Szarfman A, Waters L, Hollingdale MR, van der Meide PH, Finbloom DF, Ballou WR: **Sporozoite vaccine induces genetically restricted T-cell elimination of malaria from hepatocytes.** *Science* 1989, 244:1078–1081.

Lymphocytic infiltrates were found in the livers of immune mice after sporozoite challenge infection. Formation of these infiltrates required $CD8^+$ T cell. Immune spleen cells were able to kill malaria parasites growing in cultured liver cells only if the two cell's major histocompatibility complex genes were identical. Such T cells are probably important in killing malaria parasites in the liver.

5. • Troye-Blomberg M, Riley EM, Perlmann H, Andersson G, Larsson A, Snow RW, Allen SJ, Houghten RA, Olerup O, Greenwood BM, Perlmann P: **T- and B-cell responses of *Plasmodium falciparum* malaria-immune individuals to synthetic peptides corresponding to sequences in different regions of the *P. falciparum* antigen Pf155/RESA.** *Immunol* 1989, 143:3043–3048.

Fourteen peptides predicted to be T-cell epitopes on the Pf155/RESA molecule were tested with the lymphocytes and antibodies of malaria exposed persons. Two peptides in particular gave high rates of T-cell response, and several were B-cell epitopes. This study sets the ground work for a synthetic vaccine based on Pf155/RESA.

6. • Burns JM Jr, Marjarian WR, Young JF, Daly TM, Long CA: **A protective monoclonal antibody recognizes an epitope in the carboxyl-terminal cysteine-rich domain in the precursor of the major merozoite surface antigen of the rodent malarial parasite, *Plasmodium yoelii*.** *J Immunol* 1989, 143:2670–2676.

The epitope recognized by a protective monoclonal antibody in a rodent malaria was mapped to a cysteine-rich portion of the major merozoite surface antigen molecule. This region is similar in *P. falciparum* major merozoite surface antigen, and it is possible that protection against blood stage infections in humans might be achieved by antibodies against this epitope.

7. • Marsh K, Otoo S, Carson DC, Greenwood BM: **Antibodies to blood state antigens of *Plasmodium falciparum* in rural Gambians and their relations to protection against infection.** *Trans Roy Soc Trop Med Hyg* 1989, 83:293–303.

A cross-sectional and longitudinal study of immune responses to *P falciparum* blood stage antigens and protection from malaria. The only immune response correlating with protection against clinical illness was serum reactivity to merozoite antigens on the infected erythrocyte.

8. • Kaslow DC, Quaki IA, Keister DB: **Minimal variation in a vaccine candidate from the sexual stage of *Plasmodium falciparum*.** *Mol Biochem Parasit* 1989, 32:101–104.

Pfs25 is an antigen on the sexual stages of *P falciparum* that is a target for transmission-blocking antibodies. This gene was sequenced from eight geographically distinct isolates of *P falciparum* and little variation was found. Transmission-blocking antibodies may, thus, be effective against all strains of the parasite.

9. •• Udomsangpetch R, Aikawa M, Berzins K, Wahlgren M: **Cytoadherence of Knobless *Plasmodium falciparum*-infected erythrocytes and its inhibition by a human monoclonal antibody.** *Nature (London)* 1989, 338:763–765.

The first description of a *P. falciparum* strain that lacks surface knobs but that still binds to endothelium. Proved that the knob is not the parasite adhesion structure.

10. • Sherwood JA, Roberts DD, Spitalnik SL, Marsh K, Havery EB, Miller LH, Howard RJ: **Studies of the receptors on melanoma cells for *Plasmodium falciparum* infected erythrocytes.** *Am J Trop Med Hyg* 1989, 40:119–127.

Although purified thrombospondin binds to parasitized erythrocytes, expression of this molecule on melanoma cells did not correlate with parasite binding.

11. • Oquendo P, Hundt E, Lawler J, Seed B: **CD36 directly mediates cytoadherence of *Plasmodium falciparum* parasitized erythrocytes.** *Cell* 1989, 58:95–101.

A complementary DNA clone for the human CD36 molecule was sequenced. Tumor cells transfected with this clone bound malaria infected erythrocytes. This is strong evidence that CD36 is a ligand for the parasite adhesion molecule.

12. •• Ochenhouse CF, Tandon NJ, Magowan C, Jamieson GA, Chulay JD: **Identification of a platelet membrane glycoprotein as a falciparum malaria sequestration receptor.** *Science* 1989, 243:1469–1471.

The CD36 molecule was purified from platelets, and malaria-infected erythrocytes bound to plastic coated with this glycoprotein. Binding could be inhibited by adding soluble CD36. CD36 analogues might be useful drugs to prevent erythrocyte sequestration in patients with cerebral malaria.

13. • Grau GE, Hubertine H, Piguet PF, Pointaire P, Lambert P-H, Billiau A, Vassalli P: **Monoclonal antibody against interferon-γ can prevent experimental cerebral malaria and its associated overproduction of tumor necrosis factor.** *Proc Natl Acad Sci USA* 1989, 86:5572–5574.

In vivo treatment of mice with antibody to interferon-γ blocks production of TNF by macrophages, and prevents cerebral malaria.

14. •• Grau GE, Taylor TE, Molyneux ME, Wirima JJ, Vassalli P, Marcel H, Lambert P-H: **Tumor necrosis factor and disease severity in children with falciparum malaria.** *N Engl J Med* 1989, 320:1586–1591.

High TNF levels at hospital admission correlated with poor outcome in children with severe falciparum malaria infections.

15. • Wilson CM, Serrano AD, Wasley A, Bogenschutz MP, Shankar AH, Wirth DF: **Amplification of a gene related to mammalian mdr genes in drug-resistant *Plasmodium falciparum*.** *Science* 1984, 244:1184–1186.

Description of *P falciparum* genes homologous to the mammalian multidrug resistance gene. This may be one cause of chloroquine resistance, as a drug-resistant strain had a larger number of gene copies and a higher level of gene expression.

16. • Foote SSJ, Thompson JK, Kemp DJ: **Amplification of the multidrug resistant gene in some chloroquine-resistant isolates of *P. falciparum*.** *Cell* 1989, 57:921–930.

The gene in *P falciparum* homologous to the multidrug resistance gene in mammals has been sequenced. It is amplified in some but not all malaria strains insensitive to chloroquine and may be a mechanism of drug resistance.

17. •• PETERSON DS, WALLIKER D, WELLEMS TE. **Evidence that a point mutation in dihydrofolate reductase-thymidylate synthase confers resistance to pyrimethamine in falciparum malaria.** *Proc Natl Acad Sci U S A* 1989, 85:9114–9118.

Resistance to pyrimethamine is found to correlated with a point mutation resulting in an amino acid change at the putative binding site for the drug. This may lead to the design of more effective compounds.

18. • MOLYNEUX ME, TAYLOR TE, WIRIMA JJ, HARPER G. **Effect rate of infusion of quinine on insulin and glucose responses in Malawian children with falciparum malaria.** *Br Med J (Clin Res)* 1989, 299:602–603.

Hypoglycemia due to quinine could be avoided using slower rates of infusion.

19. •• WHITE NJ, MILLER KD, CHURCHILL FC, BERRY C, BROWN J, WILLIAMS SB, GREENWOOD BM. **Chloroquine treatment of severe malaria in children.** *N Engl J Med* 1988, 319:1493–1499.

The definitive study of the pharmacology of chloroquine use for the treatment of malaria in children. They find that the drug is rapidly absorbed by a variety of routes and can be safely given if dosing is not done too rapidly.

20. • MILLER KD, GREENBERG AE, CAMPBELL CC. **Treatment of severe malaria in the United States with a continuous infusion of quinidine gluconate and exchange transfusion.** *N Engl J Med* 1989, 321:65–70.

A review of recent cases of severe malaria in the United States known to the Centers for Disease Control. Intravenous quinidine with or without exchange transfusion was effective therapy for severe malaria.